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Form 1/77 **Patents Act 1977**

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❶ Title of invention

1 Please give the title of the invention: Vaccine

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4. Agent's or applicant's reference number *(if applicable)* RE/RH/ B45172

❺ Claiming an earlier application date

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5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

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Agent's address SmithKline Beecham
Corporate Intellectual Property
Two New Horizons Ccourt
Brentford, Middlesex

Postcode TW8 9EP

Agent's ADP number 4122317005

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Continuation sheets for this Patents Form 1/77 —

Claim(s) 2 Description 14

Abstract Drawing(s) 6 only

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Priority documents *(please state how many)*	—
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Vaccine

The present invention relates to the provision of novel peptides for the treatment, prevention or amelioration of allergic disease. In particular, the novel peptides are derived from previously unrecognised regions of IgE for both passive and active immunoprophylaxis or immunotherapy. The invention further relates to methods for their production, pharmaceutical compositions containing them and their use in medicine.

In an allergic response to allergens, the symptoms are brought about by the release of allergic mediators, such as histamine, from immune cells into the surrounding tissues and vascular structures. Histamine is normally stored in mast cells or basophils, until such time as the release is triggered by interaction with allergen specific IgE.

The role of IgE in the mediation of allergic responses, such as asthma, food allergies, atopic dermatitis, type-I hypersensitivity and sinus inflammation, is well known. On encountering an antigen, such as pollen or dust mite allergens, B cells commence the synthesis of allergen specific IgE. The allergen specific IgE then binds to its FcεRI receptor (the high affinity receptor) on basophils and mast cells. Any subsequent encounter with allergen leads to the triggering of histamine release from the mast cells or basophils, and cross-linking of neighbouring IgE/ FcεRI complexes (EP 0 477 231 B1).

IgE, like all immunoglobulins, comprises two heavy and two light chains. The ε heavy chain consists of five domains: one variable domain (VH) and four constant domains (Cε1 to Cε4). The molecular weight of IgE is about 190,000 Da, the heavy chain being 550 amino acids in length.

A number of passive or active immunotherapeutic and immunoprophylactic approaches which interfere with this IgE-mediated histamine release mechanism have been investigated. These approaches range from the prevention of allergen/IgE complexes from binding to the FcεRI or FcεRII receptors on mast cells with passively

administered antibodies, or competitive binding of IgE to the receptors by IgE derived peptides; to the use of specific IgE peptides for active immunisation to stimulate histamine release inhibiting immune responses.

There are a number of considerations, and problems, which have been encountered in the art, which have to be taken into account when designing new anti-allergy therapies. One potentially dangerous consideration revolves around the involvement of IgE cross-linking in the histamine release signal. It is most often the case that the generation of anti-IgE antibodies during active vaccination therapy, are capable of triggering histamine release *per se*, by the cross-linking of IgE in the absence of any allergens. This phenomenon is termed anaphylactogenicity. Indeed many commercially available anti-IgE monoclonal antibodies are anaphylactogenic. Whether or not an antibody is anaphylactogenic, depends on the location of the target epitope on the IgE molecule.

Therefore, in order to be safe and effective, the passively administered or vaccine induced antibodies must bind in a region which is capable of interfering with the histamine triggering pathway, without being anaphylactic *per se*. The present invention achieves these aims and provides peptides which are capable of raising non-anaphylactic antibodies which inhibit histamine release. These peptides may form the basis of an active vaccine or be used to raise appropriate antibodies for passive immunotherapy.

EP 0 477 231 B1 describes immunogens derived from the Cε4 domain of IgE (residues 497-506, also known as the Stanworth decapeptide), conjugated to Keyhole Limpet Haemocyanin (KLH) used in active vaccination immunoprophylaxis. WO 96/14333 is a continuation of the work described in EP 0 477 231 B1.

Other approaches are based on the identification of peptides which themselves compete for IgE binding to the high or low affinity receptors on basophils or mast cells (WO 93/04173, WO 93/04173, WO 98/24808, EP 0 303 625 B1, EP 0 341 290).

Additional IgE peptides conjugated to carrier molecules, are described in WO 97/31948. These immunogens may be used in vaccination studies and are said to be capable of generating antibodies which subsequently inhibit histamine release *in vivo* .

The present invention is the identification of novel sequences of IgE which are used in active or passive immunoprophylaxis or therapy. These sequences have not previously been associated with anti-allergy treatments.

The peptides of the present invention are continuous portions of IgE which have been identified as being surface exposed. They are, therefore, available for antibody recognition by auto anti-IgE antibodies. The peptides of the present invention are capable of being used in active vaccination studies to induce auto anti-IgE antibodies.

Surprisingly, the auto anti-IgE antibodies induced by the peptides of the present invention are non-anaphylactogenic and are capable of blocking IgE-mediated histamine release from mast cells and basophils.

The present invention identifies those regions of IgE which are both continuous and are solvent exposed. The peptides may be passively administered themselves to prevent histamine release, or may be administered in active prophylactic or therapeutic vaccination. Antibodies, thus produced, are active in preventing the allergic symptoms caused by histamine release from mast cells or basophils.

Additionally, antibodies induced in one animal may be purified and passively administered to another animal for the prophylaxis or therapy of allergy. The peptides of the present invention may also be used for the generation of monoclonal antibody hybridomas (using know techniques *e.g.* Köhler and Milstein, Nature, 1975, 256, p495), humanised monoclonal antibodies or CDR grafted monoclonals, by techniques known in the art. Such antibodies may be used in passive immunoprophylaxis or immunotherapy, or be used in the identification of IgE peptide mimetopes.

The IgE peptides of the present invention are:

Peptide	*Sequence*	*Length exposed*	*IgE Domain*
P1	EDGQVMDVD	9	Cε2
P2	STTQEGEL	8	Cε2
P3	SQKHWLSDRT	10	Cε2
P4	GHTFEDSTKKCADSNPRGV	19	Cε2/Cε3

P1 and P2 are located within the Cε2 domain of IgE, within a region which has not previously been reported as being useful for the active vaccination based immunoprophylaxis of allergy.

The present invention arises from the identification of these novel regions of IgE for use in allergy therapy. The present invention, therefore, includes the native IgE peptides themselves, and any mimetope thereof. The meaning of mimetope is defined as a peptide sequence which is sufficiently similar to the native IgE peptide (sequentially or structurally), which is capable of being recognised by antibodies which recognise the native IgE peptide; or are capable of raising antibodies, when coupled to a suitable carrier, which antibodies are capable of recognising the native IgE peptide.

Peptide mimetopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides may be modified for the purposes of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. Thereby reducing the conformational degrees of freedom of the peptide, and thereby increasing the probability that the peptide is presented in a conformation which most closely resembles that of the IgE peptide as found in the context of the whole IgE molecule. For example, the peptides may be altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or

substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to enhance stability of the peptide.

Examples of such modified peptides include:

Peptide	*Sequence*	*Description*
P10	CADSNPRGV	P4 variant
P15	CLEDGQVMDVDLL-NH_2	P1 variant
P16	CSTTQEGELA- NH_2	P2 variant
p17	CSQKHWLSDRT- NH_2	P3 variant

Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the IgE peptides of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native IgE peptide. This approach may have significant advantages by allowing the possibility of identifying a peptide with enhanced immunogenic properties (such as higher affinity binding characteristics to the IgE receptors or anti-IgE antibodies), or may overcome any potential self-antigen tolerance problems which may be associated with the use of the native peptide sequence. Accordingly, in one aspect of the invention there is provided mimetopes of the IgE peptides noted above.

In one embodiment of the present invention the IgE peptides are linked to carrier molecules and used as immunogens in vaccination protocols. The peptides may be linked via chemical covalent conjugation or by expression of genetically engineered fusion partners, optionally *via* a linker sequence.

The types of carriers used in the immunogens of the present invention will be readily known to the man skilled in the art. The function of the carrier is to provide cytokine

help in order to help induce an immune response against the IgE peptide. A non-exhaustive list of carriers which may be used in the present invention include: Keyhole limpet Haemocyanin (KLH), serum albumins such as bovine serum albumin (BSA), inactivated bacterial toxins such as tetanus or diptheria toxins (TT and DT), of recombinant fragments thereof (for example, Domain 1 of Fragment C of TT, or the translocation domain of DT), or the purified protein derivative of tuberculin (PPD). Alternatively the peptides may be directly conjugated to liposome carriers, which may additionally comprise immnuogens capable of providing T-cell help. Preferably the ratio of peptide to carrier is in the order of 1:1 to 1:20, more preferable between 3-15 peptides/carrier.

Chemical conjugation methods for conjugating the IgE peptides to the carrier are also well known in the art and include gluteraldehyde, and the common commercially available heterobifunctional linkers such as CDAP and SPDP (using manufacturers instructions).

In an embodiment of the invention a preferred carrier is Protein D from *Haemophilus influenzae* (EP 0 594 610 B1). Protein D is an IgD–binding protein from *Haemophilus influenzae* and has been patented by Forsgren (WO 91/18926, granted EP 0 594 610 B1). In some circumstances, for example in recombinant immunogen expression systems it may be desirable to use fragments of protein D, for example Protein D 1/3rd (comprising the N-terminal 100-110 amino acids of protein D (GB 9717953.5)).

The immunogens of the present invention may comprise the peptides as previously described, including mimetopes or analogues thereof, or may be immunologically cross-reactive derivatives or fragments thereof. Also forming part of the present invention are portions of nucleic acid which encode the immunogens of the present invention or peptides, mimetopes or derivatives thereof.

The present invention, therefore, provides novel peptides 1-9 (as defined above) for use in the manufacture of pharmaceutical compositions for the prophylaxis or therapy of allergies. Immunogens comprising the peptides and carrier molecules are also

provided for use in vaccines for the immunoprophylaxis or therapy of allergies. Accordingly, peptides or immunogens of the present invention are used in medicine, and in the medical treatment or prophylaxis of allergic disease.

IgE peptides and immunogens of the present invention may be incorporated into vaccines for the immunoprophylaxis or therapy of allergies. Vaccines of the present invention, therefore, may advantageously also include an adjuvant. Suitable adjuvants for vaccines of the present invention comprise those adjuvants that are capable of enhancing the antibody responses against the IgE peptide immunogen.

Suitable adjuvants are well known in the art (Vaccine Design – The Subunit and Adjuvant Approach, 1995, Pharmaceutical Biotechnology, Volume 6, Eds. Powell, M.F., and Newman, M.J., Plenum Press, New York and London, ISBN 0-306-44867-X). Preferred adjuvants for use with immunogens of the present invention include: aluminium or calcium salts (hydroxide or phosphate).

The vaccines of the present invention will be generally administered for both priming and boosting doses. It is expected that the boosting doses will be adequately spaced, or preferably given yearly or at such times where the levels of circulating antibody fall below a desired level. Boosting doses may consist the peptide in the absence of the original carrier molecule. Such booster constructs may comprise an alternative carrier or may be in the absence of any carrier.

In a further aspect of the present invention there is provided a vaccine as herein described for use in medicine.

The vaccine preparation of the present invention may be used to protect or treat a mammal susceptible to, or suffering from allergies, by means of administering said vaccine via systemic or mucosal route. These administrations may include injection *via* the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or *via* mucosal administration to the oral/alimentary, respiratory, genitourinary tracts. A

preferred route of administration is via the transdermal route, for example by skin pateches (PCT Appln No. PCT/US/97/21324).

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 μg of protein, preferably 1-500 μg, preferably 1-100 μg, of which 1 to 50μg is the most preferable range. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisations adequately spaced.

In a related aspect of the present invention are ligands capable of binding to the peptides of the present invention, example of such ligands are antibodies (or Fab fragments), said ligands not being IgE receptors. Also provided are the use of the ligands in medicine, and in the manufacture of medicaments for the treatment of allergies.

Also forming part of the present invention is a method of identifying peptide immunogens for the immunoprophylaxis or therapy of allergy comprising using a computer model of the structure of IgE, and identifying those peptides of the IgE which are surface exposed. These regions may then be formulated into immunogens and used in medicine.

Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Conjugation of proteins to macromolecules is disclosed by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The present invention is illustrated by but not limited to the following examples.

Examples

1.1 Peptide identification

The peptides were identified by the following technique.

The modelled structure of human IgE has been described Padlan and Davies (*Mol. Immunol.*, 23, 1063-75, 1986). Peptides were identified which were both continuous and solvent exposed. This was achieved by using Molecular Simulations software (MSI) to calculate the accessibility for each IgE amino acid, the accessible surface was averaged over a sliding window of five residues, and thereby identifying regions of the IgE peptides which had an average over that 5-mer of greater than 80Å^2.

The results of the test are shown in figure 1.

Results

From figure 1 there are a number of native peptides which may be used as immunogens for raising antibodies against IgE.

Table 1, Native surface exposed and continuous IgE peptides

Peptide Number	*Sequence*
P1	EDGQVMDVD
P2	STTQEGEL
P3	SQKHWLSDRT
P4	GHTFEDSTKKCADSNPRGV

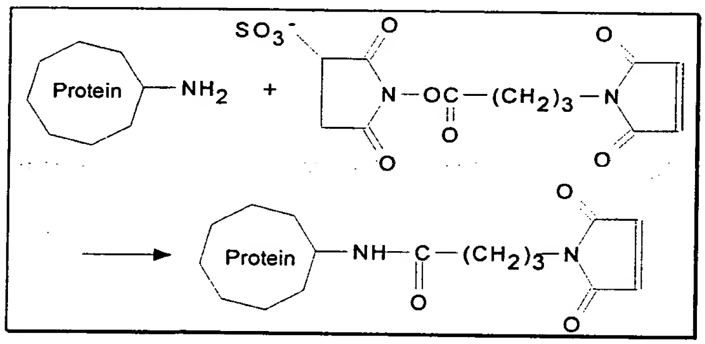
1.2 Synthesis of IgE peptide/Protein D conjugates using a succinimide-maleimide cross-linker

Protein D may be conjugated directly to IgE peptides to form antigens of the present invention by using a maleimide-succinimide cross-linker. This chemistry allows controlled NH_2 activation of carrier residues by fixing a succinimide group. Maleimide groups is a cysteine-binding site. Therefore, for the purpose of the

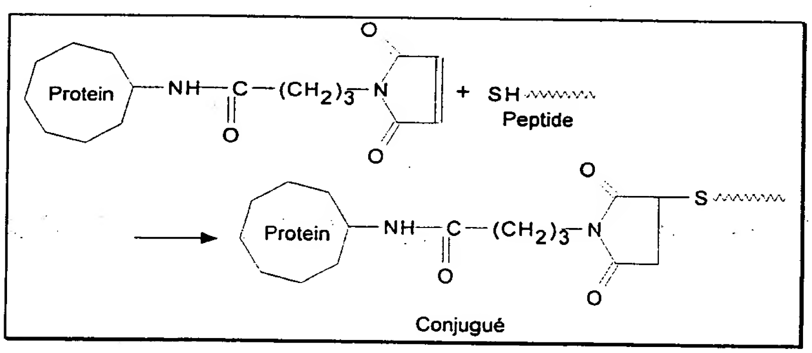
following examples, the IgE peptides to be conjugated require the addition of an N-terminal cysteine.

The coupling reagent is a selective heterobifunctional cross-linker, one end of the compound activating amino group of the protein carrier by an succinimidyl ester and the other end coupling sulhydryl group of the peptide by a maleimido group. The reactional scheme is as the following :

a. Activation of the protein by reaction between lysine and succinimidyl ester :



b. Coupling between activated protein and the peptide cysteine by reaction with the maleimido group :



1.3 Preparation of IgE peptide-Protein D conjugate

The protein D is dissolved in a phosphate buffer saline at a pH 7.2 at a concentration of 2.5 mg/ml. The coupling reagent (N-[γ-maleimidobutyryloxy] succinimide ester - GMBS) is dissolved at 102.5 mg/ml in DMSO and added to the protein solution. 1.025 mg of GMBS is used for 1 mg of Protein D. The reactional solution is incubated 1 hour at room temperature. The by-products are removed by a desalting step onto a sephacryl 200HR permeation gel. The eluant used is a phosphate buffer saline Tween 80 0.1 % pH 6.8. The activated protein is collected and pooled. The peptides (as identified in table 1 or derivatives or mimetopes thereof) is dissolved at 4 mg/ml in 0.1 M acetic acid to avoid di-sulfure bond formation. A molar ratio of between 2 to 20 peptides per 1 activated Protein D is used for the coupling. The peptide solution is slowly added to the protein and the mixture is incubated 1 h at 25°C. The pH is kept at a value of 6.6 during the coupling phase. A quenching step is performed by addition of cysteine (0.1 mg cysteine per mg of activated PD dissolved at 4 mg/ml in acetic acid 0.1 M), 30 minutes at 25°C and a pH of 6.5. Two dialysis against NaCl 150 mM Tween 80 0.1 % are performed to remove the excess of cysteine or peptide.

The last step is a sterilisant filtration on a 0.22 µm membrane. The final product is a clear filtrable solution conserved at 4°C. The final ratio of peptide/PD may be determined by amino acid analysis.

In an analogous fashion the peptides of the present invetnion may be conjugated to other carriers including BSA.

1.4 Aluminium Hydroxide adjuvant may be bought from Superfos.

1.5 ELISA assay to determine anti-antigen antibody responses

The anti-peptide and anti-carrier immune responses were investigated using an ELISA technique outlined below.

- Microtiterplates (Nunc) are coated with the specific antigen in PBS (4° overnight) with either:

Streptavidin at 2μg/ml (followed by incubation with biotinylated peptide (1μM) for 1 hour at 37°C), or

KLH, or

Protein D.

- Wash 3X PBS-Tween 20 0.1%.
- Saturate plates with PBS-BSA 1%-Tween 20 0.1% (Sat buffer) for 1 hr at 37°.
- Add 1° antibody = sera in two-step dilution (in Sat buffer), incubate 1 hr 30 minutes at 37°.
- Wash 3X.
- Add 2° anti-mouse Ig (or anti-mouse isotype specific monoclonal antibody) coupled to HRP. Incubate 1 hr at 37°.
- Wash 5X.
- Reveal with TMB (BioRad) for 10 minutes at room temperature in the dark.
- Block reaction with 0.4N H_2SO_4.

A monoclonal anti-human decapeptide antibody (Dec7B) was used as reference in deca/dodeca ELISA. This makes it possible to calculate anti-decapeptide antibody responses either in μg specific antibody per 1ml or serum (μg/ml), or as a midpoint titre. Anti-Protein D and anti-KLH responses are calculated as midpoint titers.

Example 2, *Immunisation of mice with novel IgE peptide conjugates (peptide-BSA, peptide -BSA) induces production of anti-human IgE antibodies.*

Mice can be immunised with 25μg of conjugate adsorbed onto aluminium or calcium hydroxide adjuvants.

Boosting can be performed on day 21 and on day 42 and sera can be harvested on day 42 and 56.

Anti-human IgE response can be analysed by ELISA as described below:

Microtiterplates (Nunc) are coated with 50µl chimaric human IgE anti NP (SEROTEC) 2µg/ml in PBS (4° overnight):

Wash 3X PBS-Tween 20 0.1%.

Saturate plates with 100µl PBS-BSA 1%-Tween 20 0.1% (Sat bf) for 1 hr at 37°.

Add 1° antibody = 50µl mice sera in two-step dilution (Sat bf), incubate 1 hr 30, 37°.

Wash 3X.

Add 2° antibody = 50µl anti-mouse Ig (Boehringer) coupled to HRP(5000X Sat.bf.).

Incubate 1 hr at 37°.

Wash 5X.

Reveal with 50µl TMB (BioRad) 10' at room temperature in the dark.

Block reaction with 50µl 0.4N H2SO4.

Example 3, *Anti-IgE induced in mice after immunisation with conjugate are non anaphylactogenic*

Several dilutions of complete sera or IgG purified from conjugate immunised mice can be tested in presence of basophils from freshly harvested peripheral blood from allergic patients.

The anaphylactogenicity can be evaluated by the measuring of the histamine released induced by the antibodies to be tested as described below:

- Erythrocytes are removed from peripheral blood on glucose dextran gradient
- Cells are washed and plated with samples to be tested (for example allergen, antibodies, allergen plus antibodies,...)
- After incubation , supernatants are collected and histamine release is measured according to manufacturer's instructions (Immunotech , histamine enzyme immunoassay kit)

Example 4, *Anti-IgE induced in mice after immunisation with conjugate are capable of blocking IgE mediated histamine release induced by allergen triggering of basophil from allergic patient.*

Histamine release can be measured in basophil samples triggered with various concentrations of allergen in presence or absence of several dilutions of complete sera or IgG purified from conjugate immunised mice.
Blocking activity of anti-IgE antibodies can be evaluated by the measuring of the inhibition of the histamine release induced by the allergen.
Histamine can be measured as described in example 3.

Example 5, *Anti-IgE induced in mice after immunisation with conjugate are capable of blocking local allergic response in the Monkey Cutaneous Anaphylaxis model.*

Purified anti-IgE antibodies from mice immunised according example 2 can be inoculated intradermally into the skin of a monkey sensitised with IgE.
Blocking activity of anti-IgE antibodies can be evaluated by the measuring of the inhibition of the local inflammation induced by the triggering of the IgE by the antigen.

Claims

1. A peptide selected from the group comprising:

Peptide Number	*Sequence*
P1	EDGQVMDVD
P2	STTQEGEL
P3	SQKHWLSDRT
P4	GHTFEDSTKKCADSNPRGV
P10	CADSNPRGV
P15	CLEDGQVMDVDLL-NH_2
P16	CSTTQEGELA- NH_2
p17	CSQKHWLSDRT- NH_2

or mimetopes thereof.

1. An immunogen comprising a peptide claimed in claim 1, and a carrier.
2. An immunogen as claimed in claim 2, wherein said carrier is selected from a group comprising KLH, TT, DT, BSA, PPD, OVA or protein D.
3. An immunogen as claimed in claim 3 wherein said carrier is protein D
4. A vaccine comprising a peptide as claimed in claim 1 and an adjuvant.
5. A vaccine comprising an immunogen as claimed in any one of claims 2 to 4, and an adjuvant.
6. A vaccine composition as claimed in any one of claims 6 or 7, wherein said adjuvant is selected from aluminium or calcium salts.
7. An antibody induced by a vaccine as claimed in any one of claims 5 to 7.
8. A ligand which is capable of binding to a peptide as claimed in claim 1.
9. A method of preventing or treating an allergic response comprising administering a vaccine as claimed in any one of claims 5 to 7, to an individual susceptible to or suffering from an allergic response.
10. Use of a peptide in the manufacture of a medicament for the prophylaxis or therapy of allergies.
11. A peptide or vaccine as claimed herein for use in medicine.
12. A nucleotide molecule, characterised in that said molecule encodes an peptide of claim 1.

13. A nucleotide molecule, characterised in that said molecule encodes an immunogen of claim 2.
14. A method of making an immunogen as claimed in claim 1, comprising fusing a peptide to a carrier by chemical conjugation.
15. A method of making an immunogen as claimed in claim 1, comprising fusing a gene encoding a peptide to a gene encoding a carrier and expressing said fusion in a cell.
16. A method of making a vaccine comprising manufacturing an immunogen as claims in claim 1, and formulating said immunogen with an adjuvant.

Figure 1, IgE amino acid surface exposure.

Residue	Surface Area	average/5	>50	>80
ARG_1	270.322723			
ASP_2	139.374542			
PHE_3	64.298927	117.361489	1	1
THR_4	46.170193	64.494911	1	0
PRO_5	66.64106	39.0209532	0	0
PRO_6	5.989833	29.3644934	0	0
THR_7	12.004753	21.4426604	0	0
VAL_8	16.016628	8.6200336	0	0
LYS_9	6.561028	10.2708042	0	0
ILE_10	2.527926	9.5044914	0	0
LEU_11	14.243686	13.375776	0	0
GLN_12	8.173189	19.2992118	0	0
SER_13	35.373051	29.1890154	0	0
SER_14	36.178207	32.6313328	0	0
CYS_15	51.976944	39.0100884	0	0
ASP_16	31.455273	45.6135238	0	0
GLY_17	40.066967	50.0871888	1	0
GLY_18	68.390228	46.9957994	0	0
GLY_19	58.546532	59.6253914	1	0
HIS_20	36.519997	63.4215874	1	0
PHE_21	94.603233	68.1998406	1	0
PRO_22	59.047947	65.8523506	1	0
PRO_23	92.281494	62.0824146	1	0
THR_24	46.809082	50.1635586	1	0
ILE_25	17.670317	38.6392736	0	0
GLN_26	35.008953	21.6261078	0	0
LEU_27	1.426522	12.5259452	0	0
LEU_28	7.215665	10.5518628	0	0
CYS_29	1.308269	3.5500722	0	0
LEU_30	7.799905	4.8164434	0	0
VAL_31	0	4.0672202	0	0
SER_32	7.758378	3.8055664	0	0
GLY_33	3.469549	9.5755666	0	0
TYR_34	0	20.7786542	0	0
THR_35	36.649906	28.9967052	0	0
PRO_36	56.015438	50.2230378	1	0
GLY_37	48.848633	57.590085	1	0
THR_38	109.601212	73.50021	1	0
ILE_39	36.835236	70.1846368	1	0
ASN_40	116.200531	73.2560022	1	0
ILE_41	39.437572	51.7217026	1	0
THR_42	64.20546	49.2710734	0	0
TRP_43	1.929714	35.2314448	0	0
LEU_44	24.58209	49.7665942	0	0
GLU_45	46.002388	50.9119188	1	0
ASP_46	112.113319	74.3084848	1	0
GLY_47	69.932083	91.0816862	1	1
GLN_48	118.912544	85.9516244	1	1
VAL_49	108.448097	91.6210626	1	1
MET_50	20.352079	89.4386316	1	1
ASP_51	140.46051	77.095856	1	0
VAL_52	59.019928	65.664336	1	0

	ASP_53	57.198666	72.8180802	1	0
	LEU_54	51.290497	45.9930286	0	0
	SER_55	56.1208	49.3437382	0	0
	THR_56	6.335252	47.723164	0	0
	ALA_57	75.773476	43.8934994	0	0
	SER_58	49.095795	51.656078	1	0
	THR_59	32.142174	59.4056414	1	0
	THR_60	94.933693	72.6291262	1	0
	GLN_61	45.083069	73.3905916	1	0
	GLU_62	141.8909	99.7907822	1	1
	GLY_63	52.903122	90.626043	1	1
	GLU_64	164.143127	83.4067496	1	1
	LEU_65	49.109997	57.2201384	1	0
	ALA_66	8.986602	47.5504318	0	0
	SER_67	10.957844	17.0083172	0	0
	THR_68	4.554589	7.3021006	0	0
	GLN_69	11.432554	7.2534874	0	0
	SER_70	0.578914	5.0619186	0	0
	GLU_71	8.743536	8.9567614	0	0
	LEU_72	0	10.8120506	0	0
Ca2	THR_73	24.028803	23.2812776	0	0
	LEU_74	20.709	37.264713	0	0
	SER_75	62.925049	69.375269	1	0
	GLN_76	78.660713	79.6644746	1	0
	LYS_77	160.55278	78.1594206	1	0
	HIS_78	75.474831	77.9196576	1	0
	TRP_79	13.18373	76.6092892	1	0
	LEU_80	61.726234	70.354977	1	0
	SER_81	72.108871	73.244224	1	0
	ASP_82	129.281219	81.9731098	1	1
	ARG_83	89.921066	69.9061278	1	0
	THR_84	56.828159	58.6259284	1	0
	TYR_85	1.391324	32.7696846	0	0
	THR_86	15.707874	23.8688072	0	0
	CYS_87	0	12.5031754	0	0
	GLN_88	45.416679	24.6922706	0	0
	VAL_89	0	22.314276	0	0
	THR_90	62.3368	48.4045714	0	0
	TYR_91	3.817901	45.5941352	0	0
	GLN_92	130.451477	47.9061642	0	0
	GLY_93	31.364498	62.7324992	1	0
	HIS_94	11.560145	91.805003	1	1
	THR_95	136.468475	85.5324108	1	1
	PHE_96	149.18042	87.190961	1	1
	GLU_97	99.088516	90.6138422	1	1
	ASP_98	39.657249	75.8683994	1	0
	SER_99	28.674551	62.1985202	1	0
	THR_100	62.741261	62.538042	1	0
	LYS_101	80.831024	61.892236	1	0
	LYS_102	100.786125	65.3434144	1	0
	CYS_103	36.428219	66.2248162	1	0
	ALA_104	45.930443	63.0386422	1	0
	ASP_105	67.14827	64.611715	1	0
	SER_106	64.900154	71.4769134	1	0
	ASN_107	108.651489	103.4634536	1	1

PRO_108	70.754211	99.7733764	1	1
ARG_109	205.863144	89.6122724	1	1
GLY_110	48.697884	76.8830266	1	0
VAL_111	14.094634	65.4895302	1	0
SER_112	45.00526	46.920649	0	0
ALA_113	13.786729	41.2131878	0	0
TYR_114	113.018738	48.2957174	0	0
LEU_115	20.160578	69.0320708	1	0
SER_116	49.507282	68.9550828	1	0
ARG_117	148.687027	59.4319108	1	0
PRO_118	13.401789	66.4779462	1	0
SER_119	65.402878	65.1146108	1	0
PRO_120	55.390755	40.2327528	0	0
PHE_121	42.690605	41.3511228	0	0
ASP_122	24.277737	41.6901974	0	0
LEU_123	18.993639	62.3268566	1	0
PHE_124	67.098251	96.8962368	1	1
ILE_125	158.574051	111.7612278	1	1
ARG_126	215.537506	127.2638428	1	1
LYS_127	98.602692	115.6261678	1	1
SER_128	96.506714	90.3210972	1	1
PRO_129	8.909876	47.213596	0	0
THR_130	32.048698	33.1289818	0	0
ILE_131	0	13.827639	0	0
THR_132	28.179621	23.4265788	0	0
CYS_133	0	17.1863608	0	0
LEU_134	56.904575	25.8134032	0	0
VAL_135	0.847608	29.5570474	0	0
VAL_136	43.135212	29.5570474	0	0
ASP_137	46.897842	21.0086596	0	0
LEU_138	0	41.1764274	0	0
ALA_139	14.162636	47.0972686	0	0
PRO_140	101.686447	52.0556626	1	0
SER_141	72.739418	55.9107072	1	0
LYS_142	71.689812	72.025412	1	0
GLY_143	19.275223	55.0721622	1	0
THR_144	94.73616	58.5342426	1	0
VAL_145	16.920198	49.6876606	0	0
ASN_146	90.04982	57.1762332	1	0
LEU_147	27.456902	39.7034882	0	0
THR_148	56.718086	39.9762418	0	0
TRP_149	7.372435	42.3791974	0	0
SER_150	18.283966	48.0311764	0	0
ARG_151	102.064598	61.6391216	1	0
ALA_152	55.716797	68.7565726	1	0
SER_153	124.757812	98.5254416	1	1
GLY_154	42.95969	82.7176696	1	1
LYS_155	167.128311	92.7866728	1	1
PRO_156	23.025738	92.8627182	1	1
VAL_157	106.061813	100.8782402	1	1
ASN_158	125.138039	81.2024406	1	1
HIS_159	83.0373	83.8643042	1	1
SER_160	68.749313	100.1024634	1	1
THR_161	36.335056	101.6485648	1	1
ARG_162	187.252609	105.1675772	1	1

LYS_163	132.868546	113.1483374	1	1
GLU_164	100.632362	123.7075026	1	1
GLU_165	108.653114	117.1282088	1	1
LYS_166	89.130882	118.3834944	1	1
GLN_167	154.35614	123.8092514	1	1
ARG_168	139.144974	115.0634766	1	1
ASN_169	127.761147	101.3253922	1	1
GLY_170	64.92424	72.5768498	1	0
THR_171	20.44046	47.9994236	0	0
LEU_172	10.613428	23.3896668	0	0
THR_173	16.257843	15.9639244	0	0
VAL_174	4.712363	11.9144266	0	0
THR_175	27.795528	17.8898566	0	0
SER_176	0.192971	14.6768822	0	0
THR_177	40.490578	23.6673128	0	0
LEU_178	0.192971	19.1426268	0	0
PRO_179	49.664516	25.6114614	0	0
VAL_180	5.172098	23.0737176	0	0
GLY_181	32.537144	63.9982642	1	0
THR_182	27.801859	61.8714416	1	0
ARG_183	204.815704	65.9450374	1	0
ASP_184	39.030403	67.2307834	1	0
TRP_185	25.540077	90.5017958	1	1
ILE_186	38.965874	55.8821974	1	0
GLU_187	144.156921	60.762831	1	0
GLY_188	31.717712	68.4903946	1	0
GLU_189	63.433571	60.7744084	1	0
THR_190	64.177895	42.187322	0	0
TYR_191	0.385943	36.3067554	0	0
GLN_192	51.221489	49.1596714	0	0
CYS_193	2.314879	36.401281	0	0
ARG_194	127.698151	48.626639	0	0
VAL_195	0.385943	38.7900666	0	0
THR_196	61.512733	56.894756	1	0
HIS_197	2.038627	45.6995426	0	0
PRO_198	92.838326	51.6998584	1	0
HIS_199	71.722084	59.4304494	1	0
LEU_200	30.387522	101.1251104	1	1
PRO_201	100.165688	89.5911306	1	1
ARG_202	210.511932	89.3463034	1	1
ALA_203	35.168427	111.3965334	1	1
LEU_204	70.497948	119.0851456	1	1
MET_205	140.638672	94.506279	1	1
ARG_206	138.608749	90.6326938	1	1
SER_207	87.617599	87.5937792	1	1
THR_208	15.800501	64.4796438	1	0
THR_209	55.303375	47.0393972	0	0
LYS_210	25.067995	51.8300146	1	0
THR_211	51.407516	57.0476686	1	0
SER_212	111.570686	69.0928378	1	0
GLY_213	41.888771	86.228067	1	1
PRO_214	115.529221	83.3757164	1	1
ARG_215	110.744141	72.0179718	1	0
ALA_216	37.145763	65.2725554	1	0
ALA_217	54.781963	48.4866956	0	0